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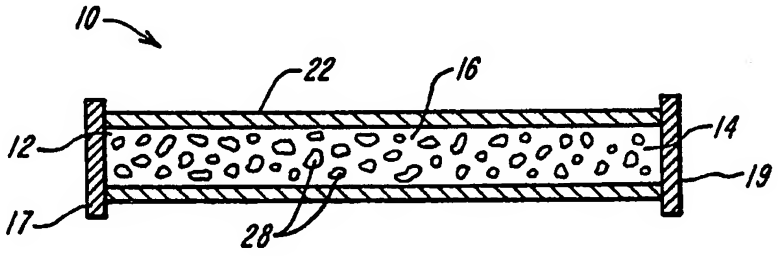


(57) Abstract

Methods and devices (10, 20, 30) are disclosed for the enhanced delivery of an active factor from an implanted, active factor-secreting cell culture to a target region in a subject. The cell culture (25) is maintained within a biocompatible, semipermeable electrically charged membrane (22) which generates electric charges on its surface, thereby resulting in enhanced delivery of active factor therefrom. The membrane permits the diffusion of active factor and metabolites therethrough, while excluding viruses, antibodies, and other detrimental agents present in the external environment from gaining access. In addition, implantable cell culture devices are disclosed which may be retrieved from the subject, replaced, or recharged with new, active factor-secreting cell cultures, and reimplanted.

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# DEVICES AND METHODS FOR ENHANCED DELIVERY OF ACTIVE FACTORS

## Background of the Invention

The technical field of this invention is the treatment of deficiency diseases and, in particular, the treatment of diseases or disorders resulting from an inadequacy or lack of a neurotransmitter, growth factor, or hormone.

Neurotransmitters, growth factors, and hormones are soluble, "trans-acting" molecules that are elicited by one cell and affect another cell. For example, neurotransmitters act as chemical means of communication between neurons. They are synthesized by the presynaptic neuron and released into the synaptic space where they are then taken up by postsynaptic neurons. Lack of neurotransmitter-mediated synaptic contact causes neuropathological symptoms, and can also lead to the ultimate destruction of the neurons involved. In fact, neurotransmitter deficits have been implicated in a number of neurological diseases.

It has been discovered that localized delivery of the relevant neurotransmitter to the target tissue may reverse the symptoms without the need for specific synaptic contact. For example, paralysis agitans, more commonly known as Parkinson's disease, is characterized by a lack of the neurotransmitter, dopamine, within the striatum of the brain, secondary to the destruction of the dopamine secreting cells of the substantia nigra. Affected subjects demonstrate a stooped posture, stiffness and slowness of movement, and rhythmic tremor of limbs, with dementia being often encountered in very advanced stages of the disease. These clinical symptoms can be improved by the systemic administration of dopamine precursors, such as L-dopa (Calne et al., (1969) Lancet ii:973-976), which are able to cross the blood-brain barrier and there to be converted into dopamine, or agonists such as bromocriptine (Calne et al., (1974) Bri. Med. J. 4:442-444) which elicit a dopamine response. Dopamine, itself, cannot be administered systemically because of its inability to cross the blood-brain barrier.

One of the drawbacks of direct systemic therapy is that other neighboring organs or tissues that respond to the administered compound are also affected. In addition, it may become increasingly difficult to administer the correct dosage of compound with time because the "therapeutic window" narrows. For example, just after L-dopa administration, the patient is overdosed, exhibiting excessive spontaneous movement; some time thereafter

the drug level may become insufficient, causing the patient to again express Parkinsonian symptoms.

Therefore, what is needed is a method of delivering an appropriate concentration of a needed compound or "active factor" to a target region of the body which is deficient in that factor.

The constitutive provision of the needed quantity of active factor has been proposed to alleviate the aforementioned problems. To this end, the transplantation of neurotransmitter- or growth factor-secreting cells has been attempted. For example, dopamine-secreting tissue has been transplanted into the brain to treat Parkinsonian symptoms. However, recent studies have shown that although the brain is considered "immuno-priviledged", rejection ultimately occurs with both allografts (identical tissue from another of the same species) and xenografts (similar tissue from another of a different species). The rejection problem necessitates the co-adminstration of immuno-suppressors, the use of which renders their own set of complications and deleterious side-effects.

To prevent the elicitation of an immune response, remedial transplantation of neuro-transmitter-secreting tissue has been tried using the patient's own tissue. For example, dopamine-secreting tissue from the adrenal medulla of patients suffering from Parkinson's disease has been implanted in their striatum with reasonable success. However, this procedure is only used in patients less than 60 years of age, as the adrenal gland of older patients

may not contain sufficient dopamine-secreting cells. This restriction limits the usefulness of this procedure as a remedy since the disease often affects older people.

An alternative way of alleviating an immune reaction in response to tissue transplantation involves protecting the cells to be implanted in a selectively permeable membrane. Such a membrane allows the diffusion therethrough of metabolites and active factors, while preventing the passage of antibodies and complement as well as viruses. However, in some systems, cell-to-cell contact is thought to be a prerequisite for the elicitation of active factor in the concentration normally required by a target tissue. In addition, diffusion rates through the membrane may change the delivery rate of an active factor from the cell culture to the target tissue.

Therefore, there exists a need for improved therapies for various deficiency diseases in general, and in particular, a need for systems which can augment or replace dysfunctional areas of an active factor-producing tissue. More specifically, there exists a need for a method of providing an active factor to a localized area of the body of a subject, the correct dosage of which will be constitutively delivered over time.

Accordingly, it is an object of the present invention to provide a method for delivering an active factor to a localized region of a subject. It is another object of the present invention to provide

a method of enhancing the delivery of active factor from encapsulated cells. Another object is to provide a method of constitutively delivering the needed dosage of an active factor to a subject deficient in that factor.

Another object is to provide an implantable cell culture device which allows for the enhanced delivery of an active factor from the cells cultured within. Yet another object is to provide a cell culture device which is retrievable, and whose contents are renewable with new and/or additional neurotransmitter-secreting cells. A further object is to provide a cell culture device which protects the cells therein from an immunological response or from viral infection, while allowing the delivery of an active factor and metabolites therefrom.

Summary of the Invention

Methods and devices are disclosed herein for the constitutive and enhanced delivery of an active factor to a subject suffering from a deficiency or dysfunction. The term "active factor" is used herein to describe neurotransmitters, growth factors, peptide hormones, trophic factors, lymphokines, and any molecule synthesized and secreted by a cell and required for the proper function and maintenance of a tissue.

It has been discovered that the delivery of an active factor from active factor-secreting cells can be greatly enhanced by encapsulating the cells in a semipermeable, electrically-charged or conductive membrane. This membrane produces surface electric charges that depolarize the encapsulated cells therein, thereby enhancing the delivery of active factor therefrom.

The term "electret" as used herein is intended to encompass natural and synthetic materials capable of generating electrical charges on their surface. Piezoelectric materials are one type of electret which generates a transient electric surface charge upon mechanical stress or deformation. Medical devices employing such electret membranes are disclosed for use in implantable cell culture devices.

The membranes of the present invention are also "semipermeable", or selectively permeable to nutrients, metabolites, and active factors having

molecular weights of about 50,000 daltons or less; excluded from passage are cells, antibodies, complement, virus and other agents harmful to the cells encapsulated therein.

In one preferred embodiment of the invention, the electret membrane is a tube composed of a piezoelectric material such as polyvinylidene difluoride (PVDF), trifluoroethylene (TrFE), or a copolymer thereof (PVDF-TrFE). Alternatively, the membrane can comprise a permanent poled polymeric material or may be fabricated in whole or in part of a conductive polymer.

In accordance with the method of present invention, at least one active factor-secreting cell, such as a neuron, is encapsulated within such a membrane and implanted into a subject where it is maintained protectively while delivering active factor to the local internal environment of that subject.

The cells to be implanted may be any cells which synthesize and secrete a desired active factor. Preferred active factors include neurotransmitters, growth factors, and active analogs, fragments, and derivatives thereof. One particularly useful active factor is the neurotransmitter, dopamine, which is secreted by cells of the adrenal medulla, embryonic ventral mesencephalic tissue, and neuroblastic cell lines such as PC12, a cell line derived from a rat pheochromacytoma. Other useful active factors include the dopamine precursor, L-dopa, and the

dopamine agonist, bromocriptine. A preferred growth factor is fibroblast growth factor (FGF) in either its acidic or basic form.

In one aspect of the invention the encapsulating membrane is a tube preferably having a diameter of about 200 - 600 mm, and a wall thickness ranging from about 50 - 100 mm. The openings of the tube may be covered by removable plugs or caps. Such a construct enables the easy replacement of cells within the membrane with other cells through the uncovered tube openings after retrieval from the subject via the attached guide wire.

Also disclosed is a method for enhancing the secretion of an active factor from an active-factor secreting cell. The method includes the steps of encapsulating the active factor-secreting cell in a semipermeable, electret membrane, and allowing the membrane to generate an electric surface charge, thereby causing the encapsulated cells to become depolarized and to secrete active factor. In a preferred embodiment, the cells is encapsulated within a piezoelectric membrane which generates the depolarizing surface charge upon mechanical stress.

Further a method for providing an active factor to a target region of the body is disclosed. In this method, an active factor-secreting cell is encapsulated within a semipermeable, electret membrane that is permeable to the active factor. The encapsulated cell is implanted in a target region of the body. Enhanced secretion of the active factor from the encapsulated cell results when the

encapsulating membrane generates a surface charge and depolarizes the cells therein.

The invention will next be described in connection with certain illustrated embodiments. However, it should be clear that various modifications, additions, and subtractions can be made without departing from the spirit or scope of the invention. The present invention should not be read to require, or be limited to, particular cell lines or electret materials described by way of sample or illustration. Additionally, although the culture devices described below are generally tubular in shape, it should be clear that various alternative shapes can be employed as well. Moreover, the electrically charged or conductive materials employed in the present invention need not form the entire encapsulating membrane but rather can be interspersed within an otherwise inactive membrane matrix.

Brief Description of the Drawings

The invention itself can be more fully understood from the following description when read together with the accompanying drawings in which:

FIG. 1 is a schematic illustration of an implantable cell culture device for delivering a active factor, according to one aspect of the invention;

FIG. 2 is a schematic illustration of an implantable and retrievable cell culture device for delivering an active factor, according to another aspect of the invention; and

FIG. 3 is a schematic illustration of an implantable, retrievable, and rechargeable cell culture device for delivering an active factor, according to yet another aspect of the invention.

Detailed Description

Methods and devices are disclosed herein for the enhanced delivery of an active factor from an implanted, active factor-secreting cell culture to a target region in a subject. This invention exploits the discovery that upon depolarization, nerve cells may be induced to release neurotransmitter from secretory granules in their cytoplasm.

In the present invention, a cell culture is maintained within a biocompatible, semipermeable electret membrane. The surface charges generated by the membrane causes the encapsulated cells therein to become depolarized and to secrete active factor. The electret membrane is also semipermeable, permitting the diffusion of active factor and metabolites therethrough, while excluding viruses, antibodies, and other detrimental agents present in the external environment from gaining access. In addition, implantable cell culture devices are disclosed which may be retrieved from the subject, replaced, or recharged with new, active factor-secreting cell cultures, and reimplanted.

Any cell line or tissue which secretes a needed active factor can be encapsulated for use in the present invention. These include tissue fragments and established cell lines that secrete active factors *in vivo* such as adrenal medulla, embryonic ventral mesencephalic tissue, neuroblastic cell lines, and PC12 cells, all of which secrete dopamine. Other useful neurotransmitters include gamma aminobutyric acid (GABA), serotonin,

acetylcholine, noradrenaline, and other compounds necessary for normal nerve functions. Preferred active factors is fibroblast growth factor in either its acidic or basic form and various peptide hormones.

In addition, any cell which secretes a precursor, analog, derivative, agonist or fragment of a desired active factor having similar activity can be used, including, for example, cells which elicit L-dopa, a precursor of dopamine, or bromocriptine, a dopamine agonist.

The cells to be encapsulated may be allografts, or cells from another of the same species as the subject in which they are to be implanted, or they may be xenografts, or those from another of a different species. They may be derived from, or are a component of an adult body organ or tissue which normally secretes a particular active factor in vivo. Alternatively, useful cells include embryonic active factor-secreting cells from, for example, the embryonic ventral mesencephalon, neuroblastoid cell lines, or the adrenal medulla.

Further, any cells which have been genetically engineered to express an active factor or its agonist, precursor, derivative, analog, or fragment thereof which has similar neurotransmitter activity are also useful in practicing this invention. Thus, in such an approach, the gene which encodes the active factor, or an analog, precursor, or fragment thereof, is either isolated from a cell line or constructed by various known engineering methods. The gene is then incorporated into a

vector, which, in turn, is transfected into a host cell for expression (see, e.g., Maniatis et al., Molecular Cloning (1982), herein incorporated by reference for further discussion of cloning vehicles and gene manipulation procedures). Appropriately transformed cells which express the active factor can be cultured in vitro until a suitable density is achieved.

The active factor-secreting cells are then placed into the cell culture device prior to implantation, either as tissue fragments or as seed cultures.

The device is a membrane adapted to receive the active-factor secreting cells. This membrane is biocompatible and semipermeable so as to protect the cells from deleterious encounters with viruses and elements of the immune system. Such protection is particularly important for preserving allografts or xenografts which are eventually considered foreign even in the "immuno-priviledged" brain. Therefore, the membrane should bar viruses, macrophages, complement, lymphocytes, and antibodies from entry while allowing the passage of nutrients, gases, metabolic breakdown products, other solutes, and the neurotransmitter to pass therethrough. Accordingly, a biocompatible and nonresorbable materials having pores enabling the diffusion therefrom of molecules having a molecular weight of up to about 50,000 daltons are useful for practicing the present invention.

Further, the membrane of the cell culture device is made of an electret material, one preferred example of which is a piezoelectric material. Useful piezoelectric membranes are composed of biocompatible, semicrystalline polymers which may have to be poled prior to use, but which need not be stretched prior to poling. Poling can then be performed to align the polymeric chain segments in a particular orientation, thereby establishing a predefined dipole moment. Polarization can be achieved, for example, by disposing one electrode on the inside of a tubular membrane and another electrode on the outside of the tube, and then applying a voltage to one of the electrodes such that an electric field is established. When the membrane is a piezoelectric material, poling preferably establishs a charge generation (polarization constant) ranging from about 0.5 - 35 picoColoumbs per Newton, and, more preferably, from about 1.0 - 20.0 picoColoumbs per Newton.

Piezoelectric materials useful in the present invention include a variety of halogenated polymers, copolymers and polymer blends. The halogenated polymers include polyvinylidene difluoride, polyvinyl fluoride, polyvinyl chloride and derivatives thereof as well as copolymers such as copolymers of the above materials and trifluoroethylene. PVDF-TrFE, for example, is a preferable material for membrane fabrication with adequate mechanical characteristics for cell filling and stereotaxic brain implantation. It need not be stretched prior to poling.

Small tubular membranes of PVDF-TrFE can be constructed by various fabrication techniques known

to those skilled in the art, including, for example, the spray-phase inversion technique. In this procedure, the tubular membrane is fabricated using a machine consisting of a small precision lathe in which mandrels of different diameters are rotated using an electronically controlled variable speed motor. A carriage is positioned adjacent to the lathe bed. The carriage can move bidirectionally and in parallel with the rotating mandrel. The carriage is driven by an electronically controlled motor which is automatically reversed by the action of electro-mechanical relays controlled by micro-switches. Two spray-guns are mounted on the carriage and can be fixed at different angles to one another, and at different distances between nozzles and mandrel, so that the jet-streams can be directed on a precise point over the mandrel.

The porosity of the membrane may be controlled by the degree of phase inversion. The advantage of PVDF-TrFE resides in its ability to be electrically poled without the need for mechanical stretching.

The cell culture device may take any shape that will accommodate the cells to be encapsulated, and which will not cause undue trauma upon surgical implantation. In addition, to ensure viability of the cells within, only small diffusion distances are established between the implanted tissue and the vascularized surrounding host tissue. To this end, the diameter of the tube should be in the range of about 200 - 600 m, as determined by the observation

that oxygen tension, when relying only on diffusion, approaches zero in a vascularized tissue.

A preferable implantable cell culture device 10 shown in FIG. 1 is a tubular, selectively permeable piezoelectric membrane 22 having ends 12 and 14 through which active factor-secreting cells 25 are loaded into cell compartment 16. Ends 12 and 14 may then be permanently occluded with caps 17 and 19 or, alternatively, with an epoxy glue or sutures of a biocompatible and nonresorbable material like polypropylene.

The device 10 as shown in FIG. 1 can be surgically implanted into the target region of a subject such that membrane 22 is in immediate contact with body tissues or fluids. The targeted region may be the in vivo site of deficiency, need, or the site of synthesis of the factor. For example, this region may be any part of the nervous system, but will most often be the brain, as it is the source of numerous neurological dysfunctions. The site will provide means for the mechanical deformation of the piezoelectric membrane, necessary for generation of the depolarizing charge. Such means includes the pulsation of adjacent blood vessels and natural movements of the body to which the membrane may be attached.

The method of the present invention may include an additional step whereby the initially encapsulated and implanted cells are retrieved from the subject in the event that they cease to produce active factor, expire, or are no longer needed to

correct the dysfunction. As illustrated in FIG. 2, retrieval of implanted cell culture device 20 can be accomplished by means of guide wire 18 which is permanently attached to end cap 17 or 19. This wire may be constructed of any nonresorbable, biocompatible material with enough tensile strength to support the cell culture device.

A exemplary cell culture device useful in practicing this method is shown in FIG. 3. Device 30 is tubular, having ends 12 and 14 reversibly covered with removable, friction-fitted caps 22 and 24, respectively, to enable the extraction and replacement of cells 25 in cell compartment 16 with new cells. The device 30 as shown in FIG. 3 can be surgically implanted into the brain of a subject such that guide wire 18 is located directly under the epithelial tissues of the head, and membrane 22 is in immediate contact with brain tissue. Transient charges will be generated by the piezoelectric membrane in response to normal movements of the head and brain.

The invention will next be described in connection with the following non-limiting examples.

## EXAMPLES

Piezoelectric semipermeable tubular membranes were fabricated on a rotating polyethylene mandrel of 500 mm diameter using a spraying-phase inversion technique. PVDF-TrFE copolymer (70:30 v/v) was obtained from Autochem, NJ. The fabrication process was undertaken in a chemical hood. A skin

was formed by spraying a PVDF-TrFE solution (1% in methyl ethyl ketone (MEK)) or cospraying a 0.5% solution of PVDF-TrFE and a 50% ethanol/water mixture onto the mandrel. Two spray-guns were operated at the same volumetric flux using compressed nitrogen as the transport gas. The spray-guns were positioned at 30°C to one another and aligned so that the jet-streams converged at a distance of 4 cm between the spray-gun nozzles and the rotating mandrel. The mandrel rotation speed and carriage movement speed were fixed at 600 rpm and 70 cm/min, respectively. Upon the completion of 16 passages, a nonsolvent solution of 1:1 $H_2O$/methanol was simultaneously but separately sprayed by the second spray-gun. The nonsolvent solution allowed the formulation of an outer porous membrane structure which functions as the inner skin structural support.

Following the fabrication process, the polyethylene mandrel was removed and immediately submerged in a bath of 1:1 $H_2O$/methanol. This step was performed to stabilize the delicate sponge-like structure of the tubular membranes by allowing the solvents to gradually leave and the nonsolvent to enter the porous structure. The tubular membranes were mechanically detached from the mandrel and dried at room temperature.

Cross-section scanning electron micrographs of the tubular membranes showed a thin inner skin surrounded by a porous network.

Tubular membrane segments (1 cm) were cut and one of their extremities capped with a fast

curing acrylic polymer glue. The tubular membranes were either left empty or filled with PC12 cells. The second extremity of the tubes was then capped with the same acrylic polymer glue.

Empty PVDF-TrFE cell culture devices implanted stereotaxically in the striatum of rats, the target structure for dopamine in Parkinson's disease, displayed a mild tissue reaction consisting primarily of microglia and reactive astrocytes.

With the cell-filled devices, intact PC12 cells were observed after 7 days in vitro. TEM micrographs showed the presence of well preserved dopamine-containing secretory granules. Intact viability of macroencapsulated PC12 cells and limited host tissue reaction to the membranes confirmed the utility of PVDF-TrFE as an encapsulation material.

We claim:

1. A medical device for use in providing an active factor to a target region of the body, said device comprising:

a semipermeable, electrically charged membrane; and

at least one active factor-secreting cell encapsulated within said membrane,

said membrane being permeable to said active factor, and impermeable to cells, virus, complement, antibodies and other factors detrimental to said cells.

2. The device of claim 1 wherein said semipermeable membrane is permeable to molecules having a molecular weight of about 50,000 daltons or less.

3. The device of claim 1 wherein said membrane comprises a tube.

4. The device of claim 3 wherein said tube has a diameter of about 200 - 600 mm.

5. The device of claim 1 wherein said membrane has a wall thickness ranging from about 50 - 100 mm.

6. The device of claim 1 wherein said membrane comprises an electret material.

7. The device of claim 1 wherein said membrane comprises a piezoelectric material.

8. The device of claim 1 wherein said membrane comprises polyvinylidene difluoride.

9. The device of claim 1 wherein said membrane comprises trifluoroethylene.

10. The device of claim 1 wherein said membrane comprises a polyvinylidene difluoride-trifluoroethylene copolymer.

11. The device of claim 1 wherein said membrane comprises a conductive polymer.

12. The device of claim 1 wherein said active factor-secreting cell is a neuron.

13. The device of claim 1 wherein said active factor is selected from the group consisting of neurotransmitters and precursors, active fragments, analogs, and derivatives thereof.

14. The device of claim 13 wherein said neurotransmitter comprises dopamine.

15. The device of claim 13 wherein said precursor comprises L-dopa.

16. The device of claim 1 wherein said active factor is selected from the group consisting of a growth factor and an active fragment, analog, and derivative thereof.

17. The device of claim 16 wherein said growth factor comprises fibroblast growth factor.

18. The device of claim 17 wherein said fibroblast growth factor comprises basic fibroblast growth factor.

19. The device of claim 18 wherein said fibroblast growth factor comprises acidic fibroblast growth factor.

20. The device of claim 1 wherein said active factor is selected from the group consisting of a hormone and an active fragment, analog, and derivative thereof.

21. A method for enhancing the secretion of an active factor from an active-factor secreting cell, said method comprising the steps of:

encapsulating said active factor-secreting cell in a semipermeable, electrically charged membrane; and

allowing said membrane to generate an electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

22. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, membrane, said membrane being permeable to molecules having a molecular weight of about 50,000 daltons or less.

23. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, electret membrane, said membrane having a substantially tubular shape.

24. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a tube having a diameter of about 200 - 600 mm.

25. The device of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a membrane having a wall thickness ranging from about 50 - 100 mm.

26. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, piezoelectric membrane, and

said allowing step further comprises subjecting said piezoelectric membrane to mechanical stress, said stressed membrane generating a transient electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

27. The device of claim 26 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, piezoelectric membrane, said membrane including a polyvinylidene difluoride-trifluroroethylene copolymer.

28. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable membrane, said membrane including a conductive polymer.

29. A method for providing an active factor to a target region of the body, said method comprising the steps of:

encapsulating an active factor-secreting cell in a semipermeable, electret membrane, said membrane being permeable to said active factor;

implanting said membrane in said target region; and

allowing said electret membrane to generate an electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

30. The method of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, membrane, said membrane being permeable to molecules having a molecular weight of about 50,000 daltons or less.

31. The method of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, electret membrane, said membrane having a substantially tubular shape.

32. The method of claim 31 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a tube having a diameter of about 200 - 600 mm.

33. The device of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a membrane having a wall thickness ranging from about 50 - 100 mm.

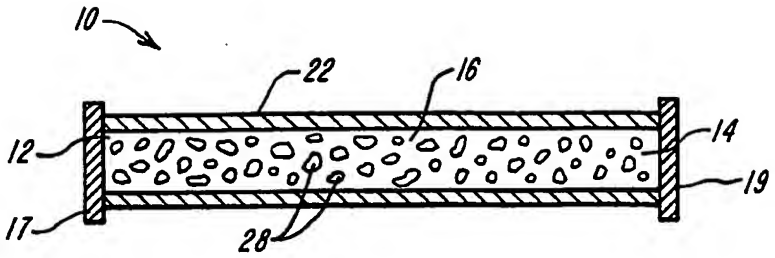


FIG. 1

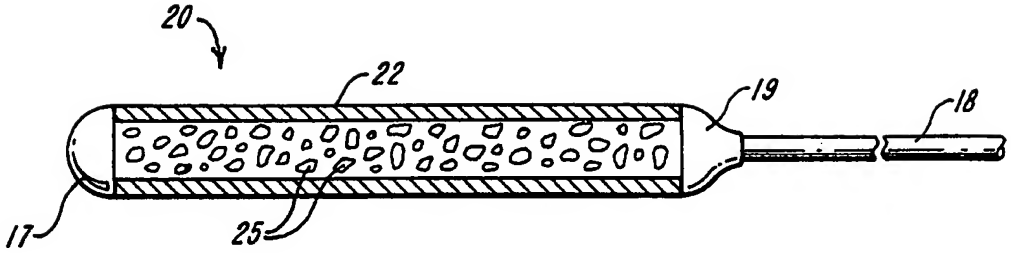


FIG. 2

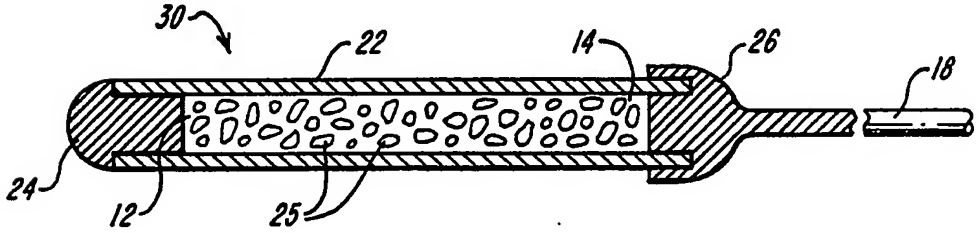


FIG. 3

# INTERNATIONAL SEARCH REPORT

International Application N PCT/US 91/00155

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 M 31/00

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC[5] | A 61 M, C 12 N |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| X | EP, A, 0127713 (GOOSEN) 12 December 1984 see abstract; page 4, lines 3-31; page 7, lines 3-34 -- | 1-6,11,21-25,28,29-33 |
| A | US, A, 4364385 (LOSSEF) 21 December 1982 see abstract; column 3, lines 1-18; column 4, lines 1-16; figure 1 -- | 1-3,6,21-23,29-31 |
| A | US, A, 4407957 (LIM) 4 October 1983 see abstract; claim 1 -- | 1-3,8,11 |
| A | GB, A, 2094833 (DAMON) 22 September 1982 see claims 1,2 -- | 1,3,6 |

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
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"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 25th April 1991 | 10. 06. 91 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | Danielle van der Haas |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 04/06/91
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